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► To cite this version:

A.C. Pfister, U. Cardini, A. Mirasole, L. Montilla, I. Veseli, et al.. Microbial associates of an endemic Mediterranean seagrass enhance the access of the host and the surrounding seawater to inorganic nitrogen under ocean acidification. *Scientific Reports*, In press. hal-04281106

HAL Id: hal-04281106

<https://hal.science/hal-04281106>

Submitted on 12 Nov 2023

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1 Title: Microbial associates of an endemic Mediterranean seagrass enhance the access of the host
2 and the surrounding seawater to inorganic nitrogen under ocean acidification

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14 *For Scientific Reports*

15

16 **Abstract**

17 Seagrasses are important primary producers in oceans worldwide. They live in shallow coastal

18 waters that are experiencing carbon dioxide enrichment and ocean acidification. *Posidonia*

19 *oceanica*, an endemic seagrass species that dominates the Mediterranean Sea, achieves high

20 abundances in seawater with relatively low concentrations of dissolved inorganic nitrogen. Here

21 we test whether microbial metabolisms associated with *P. oceanica* and surrounding seawater

22 enhance seagrass access to nitrogen. Using stable isotope enrichments of intact seagrass with

23 amino acids, we show that ammonification by free-living and seagrass-associated microbes

24 produce ammonium that is likely used by seagrass and surrounding particulate organic matter.

25 Metagenomic analysis of the epiphytic biofilm on the blades and rhizomes support the ubiquity

26 of microbial ammonification genes in this system. Further, we leveraged the presence of natural

27 carbon dioxide vents and show that microbial ammonification was reduced at lower pH. The
28 presence of *P. oceanica* enhanced the uptake of nitrogen by water column particulate organic
29 matter, increasing carbon fixation by a factor of 8.6 to 17.4 with the greatest effect at CO₂ vent
30 sites. Seagrass and its microbial associates thus enhanced water column productivity and were a
31 locus for nutrient cycling.

32 **Introduction**

33 Associations between marine species and microbes have been increasingly identified with
34 genomic methodologies, revealing many hidden species interactions between eukaryotic and
35 prokaryotic partners¹⁻³. In addition to affecting host fitness, these interactions may affect global
36 biogeochemical cycles, through the use and cycling of carbon, nitrogen and other elements^{4,5}.
37 Seagrasses are marine angiosperms and are foundational species in coastal ecosystems that are
38 critical in structuring ecosystems and modulating the flux of nutrients and energy. They provide
39 habitat for other species^{6,7}, are key primary producers^{8,9}, and also act as carbon sinks by
40 sequestering carbon^{10,11}. Marine angiosperms are some of the most widely distributed plants on
41 the planet¹², and recent studies suggest they host a diversity of microbial taxa^{13,14}, with some
42 functional partnerships demonstrated^{15,16}.

43 Among seagrasses, a number of microbial taxa have been identified as important to nutrient
44 cycling, including microbes capable of nitrogen fixation¹⁵, ammonification¹⁶ and sulfur
45 oxidation^{13,14}. *Posidonia oceanica*, the seagrass species that dominates the Mediterranean Sea,
46 achieves high abundances in coastal habitats with relatively low seawater dissolved inorganic
47 nitrogen concentrations. Microbial partnerships may partly explain its productivity. First, the low
48 oxygen environment surrounding the roots provides a microenvironment for nitrogen fixation

49 ^{15,17} and other reducing metabolisms. Second, seagrasses might have access to the dissolved
50 organic nitrogen from the surrounding seawater. A related species of *Posidonia* in Australia has
51 microbial associates that ammonify amino acids - resulting in dissolved inorganic nitrogen that is
52 taken up as ammonium ¹⁶. Although it has been claimed that seagrasses can directly take up
53 dissolved organic nitrogen sources (e.g ¹⁸), we know of no studies that show this while
54 controlling for the role of bacteria.

55 Bacteria that deaminate amino acids to produce ammonium are likely abundant in nature, given
56 that there is a diversity of enzymes that cleave carbon-nitrogen bonds producing¹⁴. Whether these
57 bacteria are consistently associated with hosts is relatively little studied, including what
58 determines their abundance. Studies that focus only on the taxonomy of microbes (e.g. amplicon
59 sequencing) do not allow strong inference about the functional capacity of microbes. Information
60 on amino acids in seawater in the environments where marine macrophytes are abundant are also
61 lacking and infrequently quantified. Nonetheless, dissolved organic compounds can be abundant
62 in coastal waters and can be a key part of nutrient recycling ¹⁹.

63 While the discovery of host-microbe interactions is still nascent, populations of many marine
64 macrophytes are in decline ^{6,20,21}. For seagrasses, global environmental change, including ocean
65 acidification and warming, pollution, and physical disturbances such as increased turbidity
66 represent key threats ²². Future ocean conditions will include a continued decrease of pH and a
67 change in carbonate system parameters. While marine angiosperms such as *P. oceanica* may
68 benefit from increased bicarbonate concentrations²³, little is known about how their associations
69 with microbes may be altered. If increased bicarbonate access improves carbon fixation and
70 fitness, host fitness might increase and microbial associates may benefit. If the availability of

71 inorganic carbon increases while nitrogen remains the same, the release of excess carbon via
72 stoichiometric overflow, could increase ²⁴, also perhaps benefitting host-associated microbes.
73 However, if low pH is a stressor to the host and host health, this may be important to host-
74 associated microbes, and microbial function and diversity might change. Microbes may also be
75 resistant or indifferent to any changes in host biogeochemistry. The seagrass *Cymodocea nodosa*
76 showed taxonomic differences in rhizome microbes but not leaf microbes across a pH gradient
77 driven by the natural release of carbon dioxide (CO₂) at vents in the Mediterranean ²⁵, though the
78 functional differences were unexplored.

79 At CO₂ vent sites in the Mediterranean the seagrass *P. oceanica* shows a number of differences
80 compared to areas without the influence of venting CO₂. Benthic communities near vents show a
81 decline in calcified species ^{26,27}, and accordingly, calcareous epiphytes of *P. oceanica* are much
82 reduced in proximity to the vents ^{8,28}. Additionally, herbivorous fish are more abundant near the
83 vents ²⁹ and *P. oceanica* has a shorter stature, fewer epiphytes, increased tissue nitrogen content
84 ³⁰, and increased shoot density ^{28,31}. Seawater pH is variable in the areas surrounding CO₂ vent
85 sites and *P. oceanica* is often in pH conditions around 7.7 ²⁷. Proximity to vents can increase *P.*
86 *oceanica* productivity at the scale of individual leaves, but shows variable results at the
87 community level ⁸.

88 The foundational role that *P. oceanica* plays in the Mediterranean ²⁹, its economic importance ³²,
89 and concern about its health status ³³ makes this species an important one to investigate host-
90 associated microbial function in a global change context, particularly under ocean acidification.
91 We thus investigated microbial function at natural CO₂ venting areas and reference areas with no
92 venting and ambient pH. We focused on nitrogen dynamics, a relatively little studied aspect of

93 ocean acidification (but see ³⁴). Populations of *P. oceanica* may have spent generations in
94 association with decreased pH, allowing tests of community interactions and functional changes,
95 including host-microbe interactions. We quantified whether ammonification, or the ability of
96 microbes to make dissolved organic nitrogen available as ammonium, occurred in *P. oceanica*
97 and if it differed in areas of low pH influenced by the vents versus areas of ambient pH.
98 Ammonification involves deamination of amino acids to liberate ammonium; we quantified
99 ammonium production in situ with stable isotope enrichments of amino acids and in the presence
100 of *Posidonia* plants and the surrounding seawater. We tested carbon fixation rates, dissolved
101 organic carbon release rates, and tissue nitrogen values as possible plant traits that differed
102 among sites. We used metagenomics to describe the microbial taxa in association with *Posidonia*
103 blades and roots, and probe their functions, both at a site with CO₂ venting with low pH and at a
104 nearby site unaffected by vent activity with ambient pH.

105 **Results**

106 The daylight carbon fixation rates per unit tissue mass of *P. oceanica* did not differ whether
107 *Posidonia* was in proximity to CO₂ vents at Castello South (CS) or in control areas with ambient
108 pH (Sant' Anna, SA) (p=0.646, Figure 1a, Table S3). At night, respiration rates too were
109 indistinguishable (Figure 1a). The nitrogen content in *Posidonia* tissue did not differ between the
110 vent and control site, either in the meristem (t=0.993, p=0.360) or midblade (t=0.517, p=0.623),
111 and averaged 2.1% in the meristem and 1.5% in the midblade region overall (Suppl Fig 1a). The
112 C:N ratio of *P. oceanica* tissue did not differ among sites, whether we considered the meristem
113 tissue or the midblade underneath epiphytes (Suppl Fig 1b). Initial conditions for the incubation
114 bottles are in Table S1; statistics and all measurements of *P. oceanica* are in Supplementary

115 Tables 2 and 3, respectively.

116 There were differences in the fate of the ^{15}N amino acids that were incubated with *P. oceanica*
117 versus seawater only, with enrichment of seawater ammonium greatest at the control site of SA
118 and greatest when in association with *P. oceanica* (Figure 2a). The ^{15}N in ammonium in seawater
119 ($\delta^{15}\text{N}$ of NH_4) increased during the first 7 daylight hours ('day') and increased again overnight
120 ('night').

121 The ammonification rate in nmol per hour across the two locales, quantified with equation (1),
122 revealed greater ammonification rates at the site with ambient pH compared with the vent site,
123 both in seawater and when *P. oceanica* was present (Figure 2b, $p=0.002$); overall,
124 ammonification rates were more than 4 times greater at ambient sites. *P. oceanica* was not
125 significantly associated with greater ammonification rates than seawater (ANOVA, $p=0.060$),
126 though ammonification with seagrass was 2.5 times greater when both sites were combined.
127 Rates did not differ between night and day ($p=0.732$). Comparing the ammonification rates
128 where *P. oceanica* was in the incubation bottles, there was no diel pattern, while seawater
129 particulate organic matter (POM) was associated with greater ammonification during the day.

130 *P. oceanica* plants showed ^{15}N enrichment in their tissues in the meristem and the midblade.
131 Enrichments were higher on the blade under epiphytes (Table S3). While all tissue types showed
132 a similar pattern, we relied on the very newest tissue growth in the meristem for analysis. There
133 was almost 3 times greater uptake of ^{15}N -enriched ammonium in the meristem during the day
134 compared to night ($p=0.002$), but the increase seen at the vent during the day switched at night to
135 greater uptake in control sites (interaction between time of the experiment and locale, $p=0.020$,

136 Figure 2c). Rates of ammonium uptake by *Posidonia* reached up to 6-8 $\mu\text{mol N}$ per g dry mass
137 per hour based on estimates of ^{15}N enrichment and using equation (2) (Figure 2c, Table S3).

138 The dynamics of dissolved organic nutrients was affected by *P. oceanica*, locale and time of day.
139 Dissolved organic carbon (DOC) was elevated when *P. oceanica* was present, with a nearly
140 significant interaction between time of day (day or night) and locale ($p=0.059$) but no significant
141 main effect of locale ($p=0.726$) or time of day ($p=0.171$) (Figure S2a). DON release in *P.*
142 *oceanica* showed no difference between locales ($p=0.580$) but was significantly elevated during
143 the day when *P. oceanica* was fixing the most carbon ($p=0.001$, Figure S2b). The pattern of
144 dissolved organic release in seawater only showed that DOC was greater at the vent site
145 ($p=0.005$) and greater at night ($p=0.041$). DON release was also greater at the vent site, though
146 the interaction between locale and time of day ($p<0.001$) indicated that DON release was greater
147 during the day at the vent site and greater at night at the control site ($p<0.001$). DOC and DON
148 tended to have net production during the day and net uptake at night. The use of inorganic
149 nutrients during daylight hours is illustrated by decreased concentrations of nitrite, nitrate and
150 silica (Figure S2).

151 The presence of *P. oceanica* was associated with increased uptake of ^{15}N in POM during
152 nighttime hours, an effect that was greatest in the control site of SA, based on the interaction
153 between locale and treatment ($p=0.033$, Figure 2d). Combining the day and night interval, POM
154 ^{15}N uptake was always greater with *P. oceanica* (Table S2), resulting in 1.6 times more nitrogen
155 uptake in chambers with *P. oceanica* compared with seawater at SA, and 2.5 times higher at the
156 CS vent site.

157 Carbon uptake by POM was also enhanced with *P. oceanica* though only during the day; POM

158 carbon uptake decreased with *P. oceanica* at night (Fig 1b). Over all estimates of POM, the C:N
159 ratio was 5.09 at SA and 5.97 at the CS vent site (Table S3). If the carbon:nitrogen uptake by
160 POM is relatively constant, water column carbon uptake was increased 8.6 times (1.6×5.09) at
161 SA and 17.4 times (2.5×5.97) at the vents due to the contribution of bacteria that metabolize
162 amino acids in association with *P. oceanica*.

163 Metagenome analyses

164 After quality control and filtering, we obtained an average of 33.7 million sequence reads per
165 sample (range 28.6 to 43.8 million). Metagenomic short reads were assembled into contigs of at
166 least 1,000 nucleotides in length, resulting in 26,460 to 123,697 contigs per sample with a mean
167 of 68,852 across all 6 samples (Table S4). Across all the metagenomes in our study, the
168 taxonomy of *Posidonia* blades had the greatest alpha diversity, with rhizomes at the vent having
169 the fewest (Figure 3, Table S4), though we are precluded from statistical tests of diversity, due to
170 lack of replication in each tissue type and locale.

171
172 The metagenomic reads allowed us to construct metagenome-assembled genomes (MAGs),
173 ranging from only a single MAG on rhizomes at the vent site to 21 MAGs on a blade
174 metagenome at the control site. We used Bowers et al ³⁵ criteria to define a MAG as high quality
175 if it had a competition score of >90% and a redundancy (or contamination) of <10% (Table S5),
176 yielding 22 high quality MAGs. A remaining 42 were medium quality MAGs that had
177 completion scores between 42% and 90% and redundancy scores between 0% and 11% (Table
178 S5). All MAGs were bacterial and represented 5 phyla (Figure 3a). A notable difference among
179 blades versus rhizomes was the presence of the Cyanobacteria and Bacteroidota phyla on blades
180 but not on rhizomes. In three cases, we were unable to taxonomically resolve a metagenome-

181 assembled genome. Taxonomic diversity at the Class and Family level was higher on rhizomes
182 compared with blades (Figure 3b,c).

183

184 The metabolism of these metagenomes was diverse, with some functional differences (Figure 4).

185 Photosynthetic and anoxygenic photosystem II were present only on blades, an expected result

186 given the presence of Cyanobacteria only on blades. Nitrogen fixation was indicated only on

187 rhizome tissue and at the control site (Figure 4). Ammonification was a prevalent metabolism

188 across all bacterial MAGs on *P. oceanica*. Of the 67 MAGs, all had enzymes classified as

189 EC:1.4.* or EC:3.5.*, while 61 MAGs had enzyme function classified as EC:4.3.1* (Table S6).

190 Nitrate reduction capability was present in the rhizome of seagrass from control areas (MAG 1

191 and MAG4), including denitrification in control rhizomes (MAG4).

192

193 Other microbial metabolisms that could be beneficial to *P. oceanica* included the presence of B

194 vitamin metabolism on both tissue types at both vent and control areas. Vitamin B6 (pyridoxal)

195 biosynthesis was only present in control rhizomes, while thiamin biosynthesis, vitamin B1, was

196 present in all samples except vent rhizomes and vitamin B7 (biotin) biosynthesis was in multiple

197 MAGS in both tissue types and locales. Vitamin B12 (cobalamin) synthesis was suggested in

198 multiple MAGs at both sites, with aerobic biosynthesis only on the blades and anaerobic

199 biosynthesis suggested in MAGs from all samples. Multiple MAGs on blades and rhizomes at

200 both locales had sulfur oxidizing capability.

201

202 **DISCUSSION**

203 *Posidonia* in proximity to CO₂ vents with low pH did not differ in carbon fixation rates from

204 those that were in ambient pH areas. Carbon fixation rates were similar to the rates estimated at
205 the same location previously⁸ for blades without epiphytes. The NPP values reported by
206 Berlinghof et al⁸ in $\mu\text{mol O}_2$ convert to ~ 0.7 to 0.9 mg C per g dry mass per hour, assuming a
207 photosynthetic quotient of 1.0 (a 1:1 molar ratio of oxygen release to carbon uptake), a range
208 nearly identical to what we estimated, though they show increased net primary production in
209 proximity to vents. Here, our estimates of primary production included the rhizome tissue which
210 may have obscured carbon fixation differences due to respiration. Ambient DIN values were
211 similar between the two sites at the single timepoint when we estimated them and previously
212 published values indicate also cite similar low nitrate concentrations regardless of venting that
213 are $<1 \mu\text{M}$ ^{36,37}. Estimates of ammonification were greater in control areas, with microbes at both
214 ambient and vent sites capable of increasing nitrogen access locally to the host. Thus, despite
215 differences in plant morphology and grazing pressure, *P. oceanica* productivity was similar.
216 Water column processes did differ between ambient and vent area, possibly due to microbial
217 nitrogen processing.

218 The presence of seagrass enhanced water column nitrogen uptake to 2.5 times at the vent site and
219 1.6 times at the ambient site when we integrated over both day and night intervals (Figure 2d).
220 With a POM C:N ratio was 5.09 at SA and 5.97 at the CS vent site (Table S3), this yields 8.6
221 times more carbon uptake at the control site and 17.4 times more carbon at the vent site due to
222 the contribution of bacteria that metabolize amino acids in association with *P. oceanica*. Direct
223 measurements of carbon uptake by POM further indicated increased carbon fixation, but only
224 during the day (Figure 1b). A possible reason for enhanced POM carbon and nitrogen uptake is
225 the increased dissolved organics available to heterotrophic bacteria when POC is present (Figure
226 S2). Nitrogen cycling in association with *P. oceanica* has been shown previously to be elevated

227 near vents ³⁴.

228 Benthic-pelagic coupling and enhancement of pelagic productivity through the host-associated
229 microbes on *P. oceanica* have implications due to global declines in seagrass cover ³⁸ and the
230 prevalence of seagrass wasting diseases ³⁹. Marine macrophytes have increasingly been shown to
231 host a species rich microbiome ⁴⁰, with diverse functions that include not only ammonification,
232 but also nitrogen fixation ^{8,34,41}, nitrification ⁴² and nitrate reduction ^{14,43}. Seagrasses are
233 important to carbon sequestration due to carbon fixation ⁴⁴ and the carbon storage in the root
234 phyllosphere ⁴⁵ and sediment. Seagrasses also ameliorate pH decreases ⁴⁶. Here we show that *P.*
235 *oceanica* enhances carbon fixation in the water column, suggesting that the contribution of
236 seagrass on the carbon cycle is likely greater than previously recognized due to water column
237 effects. *P. oceanica* mirrors the findings for other foundational species, such as corals and
238 sponges ^{4,47,48} where there are indirect effects through a food web due to the nutrient
239 provisioning by particular species. Our findings with *P. oceanica* suggests that its role as a host
240 for an active microbiome amplifies its role in these shallow Mediterranean systems.

241 Ammonification occurred during day and night and with or without *P. oceanica*, suggesting that
242 there is an abundance of microbes that metabolize amino acids and enhance carbon fixation and
243 primary productivity in seagrass ecosystems. Further, every genome that we assembled had the
244 functional capacity for deamination of amino acids to ammonium (Table S6). The use of
245 unfiltered, in situ seawater allowed us to quantify ammonification in the water column, both with
246 and without *P. oceanica*. Bacteria in the water column was likely a significant contributor to
247 ammonification. ¹⁵N uptake to water column POM was detected in all incubation bottles, though
248 an unknown amount of this uptake could be due to bacteria or eukaryotic phototrophs.

249

250 Regardless of whether the ^{15}N went into bacteria first, then into water column phytoplankton, the
251 presence of *Posidonia* enhanced the uptake of ^{15}N into POM, suggesting that seagrass-associated
252 bacteria, or even fungi, enhance water column productivity both at vent sites and controls. The
253 comparison of light versus dark uptake of ^{15}N into POM demonstrated that at least some of this
254 uptake was likely bacteria given the continued incorporation of ^{15}N into POM at night (Figure
255 2d).

256

257 One enigmatic aspect of our results is ammonification rates in units of nmol per hour, while
258 $^{15}\text{NH}_4$ uptake rates could be in μmol per hour. We can think of several explanations for this,
259 including that amino acid concentrations that were greater than the $1\mu\text{M}$ that we assumed would
260 have overestimated enrichment (R_{source}) and underestimated ammonification in equation (1). A
261 second reason is that these processes could be spatially restricted, where microbial production in
262 a biofilm could be immediately followed by host use, causing us to underestimate
263 ammonification in the water column. Thirdly, if amino acid metabolisms are rapid and amino
264 acid concentrations have high flux, we may have underestimated the processing and recycling of
265 nitrogen during incubation.

266

267 Ammonium concentrations are often not measured to the extent that nitrate concentrations are
268 measured, despite preferential use of the former by macrophytes^{49,50}. Estimates of ammonium
269 use and turnover in nature are high⁵¹, though bacterial ammonification has been measured only
270 rarely⁵². Also measured rarely is the concentration of DON, including any component parts such
271 as amino acids. Amino acid concentrations have been estimated at 0.5-1.9 μM in other areas in

272 the Mediterranean^{53,54}, but a single flux estimate in the Caribbean suggests the flux could be
273 high⁵² and the importance of DON as a persistent supply of ammonium could be even greater.

274

275 Our demonstration of increased $\delta^{15}\text{N}$ in seagrass tissue does not conclusively prove that these
276 plants are taking up $^{15}\text{NH}_4$ from microbial processes. Only pulse-chase experiments with more
277 precise imaging, for example using NanoSIMS^{15,16}, could show that microbial metabolism was
278 the intermediate between ^{15}N -amino acids and the higher $\delta^{15}\text{N}$ of macrophyte tissue. However,
279 ammonification genes were ubiquitous across MAGs here (Table S4) and in other studies^{14,55},
280 and suggests that microbes may mediate the availability of ammonium. Several previous studies
281 that suggest seagrasses directly take up DON did not control or account for microbial activity¹⁸,
282 and thus direct DON uptake by marine angiosperms remains unknown.

283

284 Amino acid concentrations in the ocean are rarely reported, though there are several studies in
285 the Mediterranean in proximity to *P. oceanica* beds that report 0.5 to 1.9 μM dissolved free
286 amino acids (DFAA) concentrations^{53,54}, several orders of magnitude greater than those reported
287 in open ocean settings^{56,57}. DFAA can be a reduced source of nitrogen for both phytoplankton
288 and bacteria¹⁹, and may be rapidly renewed via the release from animals^{58,59}, phytoplankton⁶⁰,
289 and possibly the host themselves, such as *P. oceanica* here⁵³. Concentrations can be greater in
290 the sediment surrounding *P. oceanica* when compared to the water column⁵³. Although
291 estimates are that DFAA is only from 1 to 10% of DON in coastal areas⁶¹, the estimates are few
292 and DFAA and DON may have rapid turnover rates which make their contribution to overall
293 nitrogen use difficult to estimate.

294 Our estimates of ammonium uptake by *P. oceanica* during the day from the addition of ^{15}N

295 enriched amino acids ranged from 1.17 to 15.33 umol per g dry mass per hour, comparable to the
296 2.77 umol rate estimated in ⁵¹ for *P. oceanica* in the Mediterranean and suggesting amino acids
297 from deamination may be significant to ammonium uptake rates here and in other areas.

298 *P. oceanica* has other means of acquiring dissolved inorganic nitrogen from microbial associates.
299 Nitrogen fixation genes were found in a MAG from the rhizome at the control site and is
300 consistent with other studies that have determined that nitrogen fixation ⁶², *nifH* genes ⁶³, or
301 nitrogen-fixing taxa in association with *P. oceanica* ¹⁵ in low-oxygen environment of the
302 rhizomes. Mohr et al. ¹⁵ assembled a MAG for a novel taxon that fixes nitrogen within the cells
303 of *P. oceanica* roots. We did not find the '*Candidatus Celerinatantimonas neptuna*' they
304 described in any of the MAGs we assembled, nor in the family Celerinatantimonadaceae,
305 perhaps due to either incomplete DNA sequencing or to our use of surface swabs rather than
306 grinding the root tissue of *P. oceanica*, as they did. Our analysis of *P. oceanica* blades did not
307 reveal *nifH* genes. Dissimilatory nitrate reduction to ammonium is an additional bacterial
308 metabolism that may help the seagrass host access nitrogen. We found genes for this function in
309 rhizomes in control areas, consistent with previous studies of denitrification in association with
310 seagrass⁶⁴.

311

312 Estimates of ammonification were greater in control areas, though *P. oceanica* carbon fixation
313 did not differ between the control area and the CO₂ vent area. Ambient nitrate values were
314 similar between the two sites at the single timepoint when we estimated them; previously
315 published values also cite similar low nitrate concentrations regardless of venting that are <1uM
316 ^{36,37}. Both vent and ambient sites had microbes that could increase nitrogen to enhance *P.*
317 *oceanica* primary production. In addition to the abundance of enzymes with deaminating

318 function (Table S4), nitrate reduction genes were indicated, as was nitrogen fixation.

319

320 There were microbial metabolisms that were unique to *P. oceanica* tissue types, such as
321 cyanobacteria and anoxygenic photosynthesis only on seagrass blades. B vitamin synthesis was
322 also prevalent, but the rhizomes in vent sites lacked Vitamin B1 and B6 synthesis. Vitamin B12,
323 suggested to be auxotrophic for marine eukaryotic hosts^{65,66} was in some MAGs in both tissue
324 types and locales. Whether it plays a critical role in host fitness that has been demonstrated for
325 other marine macrophytes (e.g.¹) remains to be investigated. In general, alpha diversity and the
326 number of high quality MAGs was low in vent samples. Whether this reflects lower bacterial
327 abundance in these areas is unclear, though certain functions were absent.

328

329 Our finding that *P. oceanica* and its microbial associates stimulate water column carbon fixation
330 adds an important dimension to the role of seagrass beds in the carbon cycle. Seagrasses are
331 suggested to alter the dissolved organic nitrogen⁵² and seaweeds can influence water column
332 microbes⁶⁷. Understanding whether there are general mechanisms by which the seagrass enhance
333 the productivity of the surrounding water column are a necessary aspect of quantifying the role
334 of seagrass in the marine carbon cycle.

335

336 **Methods**

337 Study System

338 Underwater CO₂ vent systems are a powerful tool for exploring the effects of low pH on the
339 features of species and biological systems. In Ischia, Italy, CO₂ vent-influenced *P. oceanica*

340 meadows (here, Castello South (CS)) is separated by less than a km from areas unaffected by
341 CO₂ vents and (Ambient pH site, Sant' Anna (SA)). The vent sites at Castello South have a mean
342 pH near 7.7, while the Ambient pH site at Sant' Anna has ambient pH ~7.95²⁷. Seawater
343 temperature and other environmental variables (e.g. depth, light, salinity) are similar in CO₂ vent
344 and in ambient pH sites²⁷. While carbon dioxide is the main gas from the vents, other gasses
345 include N₂ (3.2–6.6%), O₂ (0.6–0.8%), Ar (0.08–0.1%), and CH₄ (0.2%–0.8%) but no sulfur gas
346³¹. Trace metal concentrations can be elevated near vents, although detrimental effects on *P.*
347 *oceanica* have not been recorded⁶⁸.

348 ¹⁵N amino acid incubation with *P. oceanica*

349 *P. oceanica* was collected *in situ* at the Ambient pH site SA at 1000h and the CO₂ vent site CS at
350 1040h on 14 Sept 2021. At each site, blades attached to a single rhizome unit were selected via
351 SCUBA to have both a new blade developing as well as a corresponding older blade that was
352 generally free of epiphytes. The rhizome was removed so that the blades (from 4-6) remained
353 within a sheath. *P. oceanica* wet mass was quantified with a Pesola and ranged from 2.0 to 3.5 g
354 (mean=2.75 g). At each site, 8 *P. oceanica* shoots were collected. While we collected plants that
355 were approximately a half meter distant from each other, we do not know if individuals were
356 genetically distinct.

357 Simultaneously, gas-tight 500 ml polycarbonate bottles (actual volume is 660 ml) were filled at
358 1-2 m depth at each site, holding the bottle underwater above the *P. oceanica* beds, filling to
359 eliminate all bubbles, and capping underwater. For each site, *P. oceanica* was immediately added
360 to 8 of the bottles, while 4 held seawater only to quantify water column processes. Oxygen and
361 temperature were recorded immediately with 3 mm optical fiber probe (#OXROB3, Pyroscience,

362 Germany) and temperature sensor (Firesting FS02-4, Pyroscience, Germany) assuming 38 ppt
363 Salinity.

364 We amended each of the 16 bottles with *P. oceanica* and the 8 with seawater only with amino
365 acids that were enriched in ^{15}N (98%, Cambridge Isotope, NLM-2151, Lot#PR-24163). We
366 assumed a concentration of dissolved free amino acids (DFAA) of 1 μM based on previous work
367 in Mediterranean *Posidonia* beds that ranged from 0.5 to 1.9 μM ^{53,54}. We added 50 μL of a .05M
368 solution to an assumed existing concentration of 1 μM to achieve an enrichment of ~ 0.76 at %.
369 The bottle was gently inverted 5 times to distribute the isotope, then attached to a line with dive
370 weights at either end and were deployed at 2 m depth at noon. All 32 bottles were deployed
371 across 4 lines in the same embayment (Cartaromana Bay) where both sets of seagrasses
372 originated, though in waters that were unaffected by the vent activity. The bottles rested on the
373 benthos and thus received natural light and some gentle water surge throughout the experiment.

374 To quantify nitrogen metabolic processes during the daylight versus darkness, we analyzed half
375 of the *P. oceanica* and seawater contained within the bottles after 6 hours at 1800h; the
376 remaining 16 experimental units were removed from the water immediately after sunrise the
377 following day on 15 Sept 2021 (0720h).

378 For each of the end of day and end of night censuses, the oxygen concentration in each bottle
379 was measured and seawater was preserved for dissolved organic matter concentration, inorganic
380 nutrient concentrations, including the concentration of ^{15}N -ammonium. We also filtered 360-420
381 ml of seawater through a 0.7 μM gf/f filter (25 mm, Whatman) to quantify the ^{15}N of particulate
382 organic matter (POM) in the seawater and dried at 50°C for 48 h. Dissolved inorganic nutrient
383 samples were filtered through a 0.2 μm PE filter and frozen at -20°C. Seawater samples for

384 dissolved organic C and N analysis were filtered through pre-combusted GF/F filters into acid-
385 washed HDPE vials, immediately fixed with 160 uL of 18.5% HCl and stored at 4°C until
386 analysis on a total organic carbon analyzer (TOC-L with TNM-L Unit, Shimadzu Corporation,
387 Japan). DON was obtained from TDN subtracting total dissolved inorganic N (nitrate, nitrite and
388 ammonium), determined on seawater samples preserved frozen and analyzed on a Continuous
389 Flow Analyzer (Flowsys, SYSTEA SpA., Italy).

390 We analyzed the changes to both organic nutrient (DOC and DON) and inorganic nutrients
391 (nitrate, nitrite, ammonium, phosphorus, silica) when incubation bottles had seawater only or *P.*
392 *oceanica*, using a univariate two-way ANOVA with locale and time of day and their interaction.
393 All statistical analyses were performed in R (www.R-project.org), version 4.2.2 (2022-10-31)
394 with modules *ggplot* and *dplyr*.

395 Quantifying Nitrogen dynamics

396 We quantified the amino acid mineralization rate (ammonification) using the source-sink model
397 from Lipschultz⁶⁹. The model quantifies the transfer from the source (¹⁵N amino acids) at the
398 beginning of the experiment to the 'sink', which is the ¹⁵NH₄ in the seawater, either at the end of
399 the day or the end of the night. The rate from T_0 to T_f was based on the following difference
400 equation:

$$401 \text{ Amino Acid Mineralization Rate} = \frac{R(t)_{sink} - R(t)_{source}}{(R_{source} - R(0)_{sink}) * t} * [NH_4] \quad (\text{equation 1})$$

402 where R is the isotopic ratio of the sink or source, t is the length of the incubation, and
403 $[NH_4]$ represents the average concentration of ammonium over the course of the experiment. By
404 quantifying the metabolism of ¹⁵N from amino acids (the source) to ammonium in the water
405 column (the sink), we estimated the hourly rate in nmol for the approximately 7 hour daytime

406 incubation, as well as the entire 20 hours until the next morning. We then separated the
407 ammonification rate for the night only by assuming the $R(0)_{sink}$ was the mean value at the end of
408 the day. We quantified equation (1) for all 8 bottles with unfiltered seawater and 16 incubation
409 bottles with both *P. oceanica* and unfiltered seawater. We used unfiltered seawater from
410 immediately adjacent to the *P. oceanica* used in the experiment to quantify activity by water
411 column versus host-associated microbes.

412 Because we needed a concentration of $^{15}\text{NH}_4$ in seawater that we could detect, given the
413 relatively low concentrations of $[\text{NH}_4]$, we added 77.6 μL of .05M NH_4Cl , effectively adding 1.4
414 mg/L of NH_4 . The $\delta^{15}\text{NH}_4$ value of the NH_4Cl was known, allowing for reliable detection of
415 ammonium without diluting the $^{15}\text{NH}_4$ signal ⁵⁵.

416

417 We tracked the uptake of ^{15}N to *P. oceanica* tissue to estimate ammonium uptake rates,
418 following methods in ⁷⁰. This method uses the following equation:

419
$$\text{NH}_4^+ \text{ uptake rate} = \frac{\text{particulate } ^{15}\text{N at\% excess}}{(R * t)} * N_T \quad (\text{equation 2})$$

420

421 where tissue ^{15}N atom% excess is the final ^{15}N atom% minus the initial ^{15}N atom%, R is the
422 mean ^{15}N atom% enrichment in the NH_4^+ pool, t is the duration of the incubation, and N_T is the
423 amount of nitrogen in *P. oceanica* in μmols . We also used equation (2) to estimate ^{15}N uptake by
424 particulate organic matter (POM); for POM the ^{15}N could have been in the form of ammonium or
425 amino acids.

426

427 The percent carbon, nitrogen and $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ of *P. oceanica* tissue was measured from a 1 cm
428 section of the midblade where we scraped off all epiphytes, as well as new meristematic tissue at

429 the base that was always free of epiphytes. *P. oceanica* tissue was sampled prior to enrichment,
430 at the end of the daylight hours, and following the nighttime period. The tissue was dried, ground
431 to a fine powder in a GenoGrinder Spex (Metuchen, NJ), and analyzed on an elemental-
432 analyzer–isotope-ratio mass spectrometer at Northwestern University Stable Isotope
433 Biogeochemistry Laboratory (NUSIBL).

434

435 Metagenome Analyses

436 The surface microbiome of *P. oceanica* was sampled with a sterile cotton swab on the upper
437 rhizome tissue; the mid blade region (corresponding to 2 above) and the older tissue with
438 epiphytes (3. above) was sampled with both a cotton swab and Dentek brush to ensure a
439 complete surface sample on blade tissue. All were placed in -20°C and shipped to storage at -80°
440 C, then shipped frozen to the University of Chicago. DNA was extracted with a Qiagen
441 PowerSoil Kit and multiple samples were pooled for each of 6 metagenome samples to increase
442 DNA quantity and possible discovery. The concentration of DNA was less on Castello South
443 (CS) vent site samples compared with the non-venting site (SA), so we pooled all 6 sampled
444 individuals into a single sample for each of the vent blade and vent rhizome. Thus, *P. oceanica*
445 blade samples were pooled from 6 plants at CS and 3 plants each at SA; rhizome samples were
446 also pooled from 6 plants at CS and 3 plants each at SA. We thus had duplicated samples for
447 control blades and rhizomes but only a single sample for CS vent tissue type.

448

449 The above 6 samples were run over 1 lane on a HiSeq 2500 (2x150) with TruSeq DNA library
450 preps at Argonne National Laboratory. For each sample, resulting DNA sequences were first
451 quality filtered ⁷¹, then assembled with IDBA-UD v1.1.3 ⁷² with a minimum scaffold length of 1

452 kbp. Metagenomic short reads from each sample were then recruited back to their corresponding
453 assembled contigs using Bowtie2⁷³. Samtools⁷⁴ was used to generate sorted and indexed BAM
454 files. Anvi'o v7.0⁷⁵ was used as the command line environment for all downstream analyses.
455 'anvi-gen-contigs-database' was used to generate anvi'o contigs databases, during which
456 Prodigal v2.6.3⁷⁶ identified open reading frames, and 'anvi-run-hmms' was used to identify
457 genes matching to archaeal and bacterial single-copy core gene collections using HMMER⁷⁷.
458 We quantified overall alpha diversity with 'anvi-estimate-scg-taxonomy'. We then reconstructed
459 genomes from the assembled metagenomes, we used a combination of automatic binning via
460 CONCOCT v1.1.0⁷⁸, followed by a manual curation of each MAG that had greater than 50%
461 completion, following guidance by⁷⁹ Genome taxonomy was determined using the GTDB
462 (v.1.3.0,⁸⁰) database and 'anvi-run-scg-taxonomy'. We also inferred gene-level taxonomy using
463 Centrifuge v1.0.4⁸¹ to aid manual curation.

464

465 We examined the metabolic capability of the bacterial MAGs associated with *P. oceanica* using
466 'anvi-estimate-metabolism' on the manually refined bins that were >50% complete and <10%
467 redundancy⁸². We classified a metabolic module as complete if 75% percent of the genes within
468 the module were complete.

469

470 We further quantified the presence of genes that could increase host access to nitrogen. Our
471 experiment specifically tested whether amino acid deamination occurred, so we searched within
472 our assembled MAGs for ammonification hydrolases, including ureases and ammonia-lyases that
473 cleave the C-N bonds in amino acids and make ammonium available to the host. We identified
474 these enzymes through the Enzyme Commission numbers, and searched for EC numbers

475 classified as acting on CH-NH₂ bonds (EC:1.4.*), acting on carbon-nitrogen bonds other than
476 peptides (EC:3.5.*), or ammonia lyases (EC:4.3.1*), where * indicates any subset of these
477 classifications, and determined whether a MAG had each of these 3 classes of enzymes (e.g. ¹⁴).
478 The Kegg Orthologs associated with each of these EC numbers is shown in Supplemental Table
479 5. We searched for evidence of nitrogen fixation genes (*nifH*), as well as other nitrogen cycling
480 genes and functions that could benefit the host.

481

482 **References**

- 483 1. Croft, M. T., Lawrence, A. D., Raux-Deery, E., Warren, M. J. & Smith, A. G. Algae acquire
484 vitamin B12 through a symbiotic relationship with bacteria. *Nature* **438**, 90–93 (2005).
- 485 2. Matthews, J. L. *et al.* Symbiodiniaceae-bacteria interactions: rethinking metabolite exchange
486 in reef-building corals as multi-partner metabolic networks. *Environ Microbiol* **22**, 1675–
487 1687 (2020).
- 488 3. Wichard, T. *et al.* The green seaweed *Ulva*: a model system to study morphogenesis. *Front.*
489 *Plant Sci.* **6**, (2015).
- 490 4. de Goeij, J. M. *et al.* Surviving in a marine desert: The sponge loop retains resources within
491 coral reefs. *Science* **342**, 108–110 (2013).
- 492 5. Dittami, S. M. *et al.* A community perspective on the concept of marine holobionts: current
493 status, challenges, and future directions. *PeerJ* **9**, e10911 (2021).
- 494 6. Duffy, J. E. *et al.* Toward a Coordinated Global Observing System for Seagrasses and
495 Marine Macroalgae. *Front. Mar. Sci.* **6**, 317 (2019).
- 496 7. Shelton, A. O. Temperature and community consequences of the loss of foundation species:
497 Surfgrass (*Phyllospadix* spp., Hooker) in tidepools. *Journal of Experimental Marine Biology and*

- 498 *Ecology* **391**, 35–42 (2010).
- 499 8. Berlinghof, J. *et al.* The role of epiphytes in seagrass productivity under ocean acidification.
500 *Sci Rep* **12**, 6249 (2022).
- 501 9. Pfister, C. A., Altabet, M. A. & Weigel, B. L. Kelp beds and their local effects on seawater
502 chemistry, productivity, and microbial communities. *Ecology* (2019) doi:10.1002/ecy.2798.
- 503 10. Duarte, C. M. Reviews and syntheses: Hidden forests, the role of vegetated coastal habitats
504 in the ocean carbon budget. *Biogeosciences* **14**, 301–310 (2017).
- 505 11. Duarte, C. M., Losada, I. J., Hendriks, I. E., Mazarrasa, I. & Marbà, N. The role of coastal
506 plant communities for climate change mitigation and adaptation. *Nature Clim Change* **3**,
507 961–968 (2013).
- 508 12. Pfister, C. A. The evolutionary past and the uncertain future of foundational species. *Proc.*
509 *Natl. Acad. Sci. U.S.A.* **119**, e2211134119 (2022).
- 510 13. Crump, B. C., Wojahn, J. M., Tomas, F. & Mueller, R. S. Metatranscriptomics and Amplicon
511 Sequencing Reveal Mutualisms in Seagrass Microbiomes. *Frontiers in Microbiology* **9**,
512 (2018).
- 513 14. Miranda, K. *et al.* The Diversity and Functional Capacity of Microbes Associated with
514 Coastal Macrophytes. *mSystems* e00592-22 (2022) doi:10.1128/msystems.00592-22.
- 515 15. Mohr, W. *et al.* Terrestrial-type nitrogen-fixing symbiosis between seagrass and a marine
516 bacterium. *Nature* (2021) doi:10.1038/s41586-021-04063-4.
- 517 16. Tarquinio, F. *et al.* Microorganisms facilitate uptake of dissolved organic nitrogen by
518 seagrass leaves. *The ISME Journal* **12**, 2796–2800 (2018).
- 519 17. Agawin, N. *et al.* Significant nitrogen fixation activity associated with the phyllosphere of
520 Mediterranean seagrass *Posidonia oceanica*: first report. *Mar. Ecol. Prog. Ser.* **551**, 53–62

- 521 (2016).
- 522 18. Vonk, J. A., Middelburg, J. J., Stapel, J. & Bouma, T. J. Dissolved organic nitrogen uptake
523 by seagrasses. *Limnol. Oceanogr.* **53**, 542–548 (2008).
- 524 19. Bronk, D. A., See, J. H., Bradley, P. & Killberg, L. DON as a source of bioavailable nitrogen
525 for phytoplankton. *Biogeosciences* **4**, 283–296 (2007).
- 526 20. Evans, S. M., Griffin, K. J., Blick, R. A. J., Poore, A. G. B. & Vergés, A. Seagrass on the
527 brink: Decline of threatened seagrass *Posidonia australis* continues following protection.
528 *PLoS ONE* **13**, e0190370 (2018).
- 529 21. Krumhansl, K. A. *et al.* Global patterns of kelp forest change over the past half-century.
530 *Proceedings of the National Academy of Sciences* **113**, 13785–13790 (2016).
- 531 22. van Katwijk, M. M., Bos, A. R., Kennis, P. & de Vries, R. Vulnerability to eutrophication of
532 a semi-annual life history: A lesson learnt from an extinct eelgrass (*Zostera marina*)
533 population. *Biological Conservation* **143**, 248–254 (2010).
- 534 23. Russell, B. D. *et al.* Future seagrass beds: Can increased productivity lead to increased
535 carbon storage? *Marine Pollution Bulletin* **73**, 463–469 (2013).
- 536 24. Weigel, B. L. & Pfister, C. A. The dynamics and stoichiometry of dissolved organic carbon
537 release by kelp. *Ecology* **102**, (2021).
- 538 25. Banister, R. B., Schwarz, M. T., Fine, M., Ritchie, K. B. & Muller, E. M. Instability and
539 Stasis Among the Microbiome of Seagrass Leaves, Roots and Rhizomes, and Nearby
540 Sediments Within a Natural pH Gradient. *Microb Ecol* (2021) doi:10.1007/s00248-021-
541 01867-9.

- 542 26. Kroeker, K. J., Micheli, F., Gambi, M. C. & Martz, T. R. Divergent ecosystem responses
543 within a benthic marine community to ocean acidification. *Proc. Natl. Acad. Sci. U.S.A.* **108**,
544 14515–14520 (2011).
- 545 27. Teixidó, N. *et al.* Functional biodiversity loss along natural CO₂ gradients. *Nat Commun* **9**,
546 5149 (2018).
- 547 28. Mecca, S., Casoli, E., Ardizzone, G. & Gambi, M. C. Effects of ocean acidification on
548 phenology and epiphytes of the seagrass *Posidonia oceanica* at two CO₂ vent systems of
549 Ischia (Italy). *Medit. Mar. Sci.* **21**, 70 (2020).
- 550 29. Mirasole, A., Badalamenti, F., Di Franco, A., Gambi, M. C. & Teixidó, N. Boosted fish
551 abundance associated with *Posidonia oceanica* meadows in temperate shallow CO₂ vents.
552 *Science of The Total Environment* **771**, 145438 (2021).
- 553 30. Scartazza, A. *et al.* Carbon and nitrogen allocation strategy in *Posidonia oceanica* is altered
554 by seawater acidification. *Science of The Total Environment* **607–608**, 954–964 (2017).
- 555 31. Foo, S. A., Byrne, M., Ricevuto, E. & Gambi, M. C. The Carbon Dioxide Vents of Ischia,
556 Italy, A Natural System to Assess Impacts of Ocean Acidification on Marine Ecosystems:
557 An Overview of Research and Comparisons with Other Vent Systems. in *Oceanography and*
558 *Marine Biology* (eds. Hawkins, S. J., Evans, A. J., Dale, A. C., Firth, L. B. & Smith, I. P.)
559 237–310 (CRC Press, 2018). doi:10.1201/9780429454455-4.
- 560 32. Jackson, E. L., Rees, S. E., Wilding, C. & Attrill, M. J. Use of a seagrass residency index
561 to apportion commercial fishery landing values and recreation fisheries expenditure to
562 seagrass habitat service: Seagrass Contribution to Fishery Value. *Conservation Biology* **29**,
563 899–909 (2015).
- 564 33. Telesca, L. *et al.* Seagrass meadows (*Posidonia oceanica*) distribution and trajectories of

- 565 change. *Sci Rep* **5**, 12505 (2015).
- 566 34. Berlinghof, J. *et al.* Accelerated nitrogen cycling on seagrass leaves in a high-CO₂ world. *in*
567 *review*.
- 568 35. The Genome Standards Consortium *et al.* Minimum information about a single amplified
569 genome (MISAG) and a metagenome-assembled genome (MIMAG) of bacteria and archaea.
570 *Nat Biotechnol* **35**, 725–731 (2017).
- 571 36. Kumar, A. *et al.* Ocean acidification affects biological activities of seaweeds: A case study of
572 *Sargassum vulgare* from Ischia volcanic CO₂ vents. *Environmental Pollution* **259**, 113765
573 (2020).
- 574 37. Lorenti, M., Buia, M. C., Di Martino, V. & Modigh, M. Occurrence of mucous aggregates
575 and their impact on *Posidonia oceanica* beds. *Science of The Total Environment* **353**, 369–
576 379 (2005).
- 577 38. Marbà, N., Díaz-Almela, E. & Duarte, C. M. Mediterranean seagrass (*Posidonia oceanica*)
578 loss between 1842 and 2009. *Biological Conservation* **176**, 183–190 (2014).
- 579 39. Groner, M. *et al.* Warming sea surface temperatures fuel summer epidemics of eelgrass
580 wasting disease. *Mar. Ecol. Prog. Ser.* **679**, 47–58 (2021).
- 581 40. Park, J., Davis, K., Lajoie, G. & Parfrey, L. W. Alternative approaches to identify core
582 bacteria in *Fucus distichus* microbiome and assess their distribution and host-specificity.
583 *Environmental Microbiome* **17**, 55 (2022).
- 584 41. Capone, D. G. Nitrogen Fixation (Acetylene Reduction) by Rhizosphere Sediments of the
585 Eelgrass *Zostera marina*. *Marine Ecology Progress Series* **10**, 67–75 (1982).
- 586 42. Capone, D. G. & Taylor, B. F. MICROBIAL NITROGEN CYCLING IN A SEAGRASS
587 COMMUNITY. *in Estuarine Perspectives* (ed. KENNEDY, V. S.) 153–161 (Academic

- 588 Press, 1980). doi:10.1016/B978-0-12-404060-1.50021-6.
- 589 43. Weigel, B. L., Miranda, K. K., Fogarty, E. C., Watson, A. R. & Pfister, C. A. Functional
590 Insights into the Kelp Microbiome from Metagenome-Assembled Genomes. *mSystems*
591 e01422-21 (2022) doi:10.1128/msystems.01422-21.
- 592 44. Duarte, C. M. & Krause-Jensen, D. Export from Seagrass Meadows Contributes to Marine
593 Carbon Sequestration. *Frontiers in Marine Science* **4**, (2017).
- 594 45. Sogin, E. M. *et al.* Sugars dominate the seagrass rhizosphere. *Nat Ecol Evol* (2022)
595 doi:10.1038/s41559-022-01740-z.
- 596 46. Hendriks, I. E. *et al.* Photosynthetic activity buffers ocean acidification in seagrass meadows.
597 *Biogeosciences* **11**, 333–346 (2014).
- 598 47. Allgeier, J. E., Burkepille, D. E. & Layman, C. A. Animal pee in the sea: consumer-mediated
599 nutrient dynamics in the world’s changing oceans. *Glob Change Biol* **23**, 2166–2178 (2017).
- 600 48. Rådecker, N., Pogoreutz, C., Voolstra, C. R., Wiedenmann, J. & Wild, C. Nitrogen cycling
601 in corals: the key to understanding holobiont functioning? *Trends in Microbiology* **23**, 490–
602 497 (2015).
- 603 49. Bracken, M. E. S. & Stachowicz, J. J. Seaweed diversity enhances nitrogen uptake via
604 complementary use of nitrate and ammonium. *Ecology* **87**, 2397–2403 (2006).
- 605 50. Herbert, R. A. Nitrogen cycling in coastal marine ecosystems. *FEMS Microbiol Rev* **23**, 563–
606 590 (1999).
- 607 51. Lepoint, G., Gobert, S., Dauby, P. & Bouquegneau, J.-M. Contributions of benthic and
608 planktonic primary producers to nitrate and ammonium uptake fluxes in a nutrient-poor
609 shallow coastal area (Corsica, NW Mediterranean). *Journal of Experimental Marine Biology*
610 *and Ecology* **302**, 107–122 (2004).

- 611 52. Smith, G. W. Influence of microbial deamination on ammonium pools in marine waters.
612 *Science of The Total Environment* **75**, 319–324 (1988).
- 613 53. Jørgensen, N. O. G., Blackburn, T. H., Henriksen, K. & Bay, D. The Importance of
614 *Posidonia oceanica* and *Cymodocea nodosa* as Contributors of Free Amino Acids in Water
615 and Sediment of Seagrass Beds. *Marine Ecology* **2**, 97–112 (1981).
- 616 54. Velimirov, B. DOC dynamics in a Mediterranean seagrass system. *Marine Ecology Progress*
617 *Series* **28**, 21–41 (1986).
- 618 55. Hochroth, A. & Pfister, C. A. Microbial Processing of Organic Nitrogen by Microbes
619 Facilitates Host Nitrogen Uptake. *in review*.
- 620 56. Keil, R. G. & Kirchman, D. L. Dissolved combined amino acids: Chemical form and
621 utilization by marine bacteria. *Limnology and Oceanography* **38**, 1256–1270 (1993).
- 622 57. Suttle, C. A., Chan, A. M. & Fuhrman, J. A. Dissolved free amino acids in the Sargasso Sea:
623 uptake and respiration rates, turnover times, and concentrations. *Marine Ecology Progress*
624 *Series* **70**, 189–199 (1991).
- 625 58. Maas, A. E. *et al.* Migratory Zooplankton Excreta and Its Influence on Prokaryotic
626 Communities. *Front. Mar. Sci.* **7**, 573268 (2020).
- 627 59. Nagata, T. & Kirchman, D. L. Release of dissolved free and combined amino acids by
628 bacterivorous marine flagellates. *Limnol. Oceanogr.* **36**, 433–443 (1991).
- 629 60. Sarmiento, H. *et al.* Phytoplankton species-specific release of dissolved free amino acids and
630 their selective consumption by bacteria. *Limnol. Oceanogr.* **58**, 1123–1135 (2013).
- 631 61. Sipler, R. E. & Bronk, D. A. Dynamics of Dissolved Organic Nitrogen. in *Biogeochemistry*
632 *of Marine Dissolved Organic Matter* 127–232 (Elsevier, 2015). doi:10.1016/B978-0-12-
633 405940-5.00004-2.

- 634 62. Agawin, N. S. R., Ferriol, P., Sintes, E. & Moyà, G. Temporal and spatial variability of in
635 situ nitrogen fixation activities associated with the Mediterranean seagrass *Posidonia*
636 *oceanica* meadows: *Nitrogen fixation in Posidonia oceanica*. *Limnol. Oceanogr.* **62**, 2575–
637 2592 (2017).
- 638 63. Garcias-Bonet, N., Arrieta, J. M., Duarte, C. M. & Marbà, N. Nitrogen-fixing bacteria in
639 Mediterranean seagrass (*Posidonia oceanica*) roots. *Aquatic Botany* **131**, 57–60 (2016).
- 640 64. D., W. *et al.* Denitrification in an intertidal seagrass meadow, a comparison of ¹⁵N-isotope
641 and acetylene-block techniques: dissimilatory nitrate reduction to ammonia as a source of N
642 ²O? *Marine Biology* **139**, 1029–1036 (2001).
- 643 65. Brawley, S. H. *et al.* Insights into the red algae and eukaryotic evolution from the genome of
644 *Porphyra umbilicalis* (Bangiophyceae, Rhodophyta). *Proc Natl Acad Sci USA* **114**, E6361–
645 E6370 (2017).
- 646 66. Helliwell, K. E., Wheeler, G. L., Leptos, K. C., Goldstein, R. E. & Smith, A. G. Insights into
647 the Evolution of Vitamin B12 Auxotrophy from Sequenced Algal Genomes. *Molecular*
648 *Biology and Evolution* **28**, 2921–2933 (2011).
- 649 67. Chen, M. Y. & Parfrey, L. W. Incubation with macroalgae induces large shifts in water
650 column microbiota, but minor changes to the epibiota of co-occurring macroalgae.
651 *Molecular Ecology* **27**, 1966–1979 (2018).
- 652 68. Mishra, A. K., Santos, R. & Hall -Spencer, J. M. Elevated trace elements in sediments and
653 seagrasses at CO₂ seeps. *Marine Environmental Research* **153**, 104810 (2020).
- 654 69. Lipschultz, F. Isotope tracer methods for studies of the marine nitrogen cycle. in *Nitrogen in*
655 *the Marine Environment (2nd Edition)* (eds. Capone, D. G., Bronk, D. A., Mulholland, M. R.
656 & Carpenter, E. J.) 1345–1384 (Academic Press, 2008).

- 657 70. Pather, S., Pfister, C. A., Post, D. M. & Altabet, M. A. Ammonium cycling in the rocky
658 intertidal: Remineralization, removal, and retention. *Limnology and Oceanography* **59**, 361–
659 372 (2014).
- 660 71. Minoche, A. E., Dohm, J. C. & Himmelbauer, H. Evaluation of genomic high-throughput
661 sequencing data generated on Illumina HiSeq and Genome Analyzer systems. *Genome Biol*
662 **12**, R112 (2011).
- 663 72. Peng, Y., Leung, H. C. M., Yiu, S. M. & Chin, F. Y. L. IDBA-UD: a de novo assembler for
664 single-cell and metagenomic sequencing data with highly uneven depth. *Bioinformatics* **28**,
665 1420–1428 (2012).
- 666 73. Langmead, B. & Salzberg, S. L. Fast gapped-read alignment with Bowtie 2. *Nat Methods* **9**,
667 357–359 (2012).
- 668 74. Li, H. *et al.* The sequence alignment/map format and SAMtools. *Bioinformatics* **25**, 2078–
669 2079 (2009).
- 670 75. Eren, A. M. *et al.* Community-led, integrated, reproducible multi-omics with anvi'o. *Nat*
671 *Microbiol* **6**, 3–6 (2021).
- 672 76. Hyatt, D. *et al.* Prodigal: prokaryotic gene recognition and translation initiation site
673 identification. *BMC Bioinformatics* **11**, 119 (2010).
- 674 77. Eddy, S. R. Accelerated Profile HMM Searches. *PLoS Comput Biol* **7**, e1002195 (2011).
- 675 78. Alneberg, J. *et al.* Binning metagenomic contigs by coverage and composition. *Nat Methods*
676 **11**, 1144–1146 (2014).
- 677 79. Shaiber, A. *et al.* Functional and genetic markers of niche partitioning among enigmatic
678 members of the human oral microbiome. *Genome Biol* **21**, 292 (2020).

- 679 80. Parks, D. H. *et al.* GTDB: an ongoing census of bacterial and archaeal diversity through a
680 phylogenetically consistent, rank normalized and complete genome-based taxonomy. *Nucleic*
681 *Acids Research* gkab776 (2021) doi:10.1093/nar/gkab776.
- 682 81. Kim, D., Song, L., Breitwieser, F. P. & Salzberg, S. L. Centrifuge: rapid and sensitive
683 classification of metagenomic sequences. *Genome Res.* **26**, 1721–1729 (2016).
- 684 82. The Genome Standards Consortium *et al.* Minimum information about a single amplified
685 genome (MISAG) and a metagenome-assembled genome (MIMAG) of bacteria and archaea.
686 *Nat Biotechnol* **35**, 725–731 (2017).
- 687 83. Delmont, T. O. *et al.* Heterotrophic bacterial diazotrophs are more abundant than their
688 cyanobacterial counterparts in metagenomes covering most of the sunlit ocean. *ISME J* **16**,
689 927–936 (2022).

690

691 **Acknowledgements**

692 We thank the University of Chicago, France and Chicago Collaborating in the Sciences
693 (FAACTS) and the National Research Agency Investments for the Future (“4Oceans-Make Our
694 Planet Great Again” grant, ANR-17-MOPGA-001) for funding. We thank Pietro Sorvino and
695 family for hospitality and logistical support throughout the study. S. Owens and S. Greenwald at
696 Argonne National Lab facilitated metagenome sequencing. We thank Francesca Margiotta for
697 the inorganic and organic nutrient analyses and J. Berlinghof for comments.

698

699 **Author Contributions**

700 CAP, NT, UC, AM, JPG designed the study and CAP, NT, UC, AM, LM carried out all field and
701 laboratory analyses. CAP, UC, IV analyzed data. All individuals contributed to the writing of the

702 manuscript.

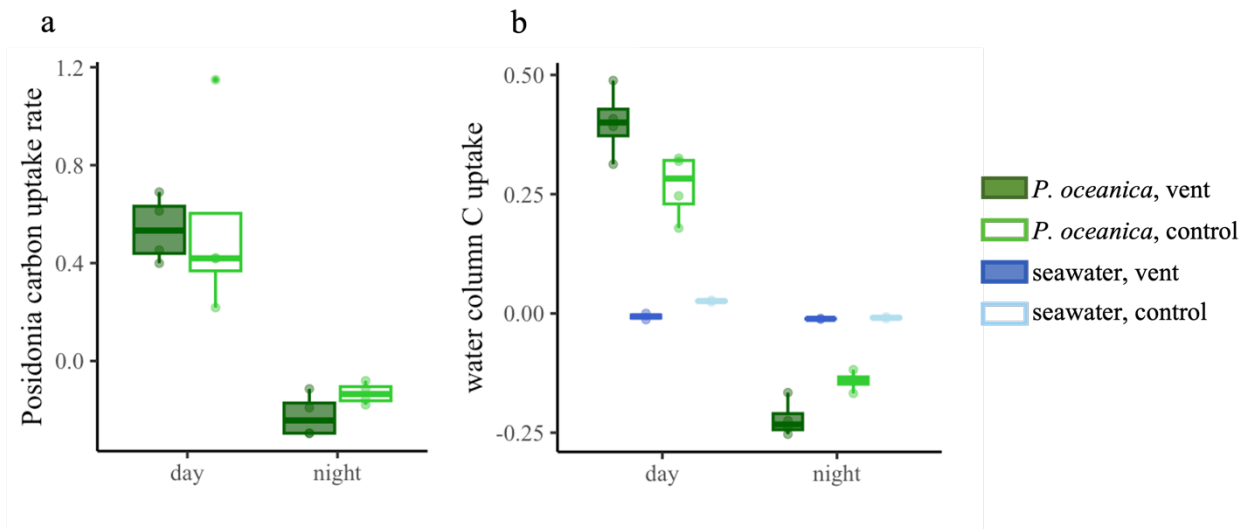
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704 **Data Availability**

705 Environmental data have been archived at Zenodo, doi: 10.5281/zenodo.7834043, while

706 metagenomic data are at NCBI, #SUB13085574.

Figure 1

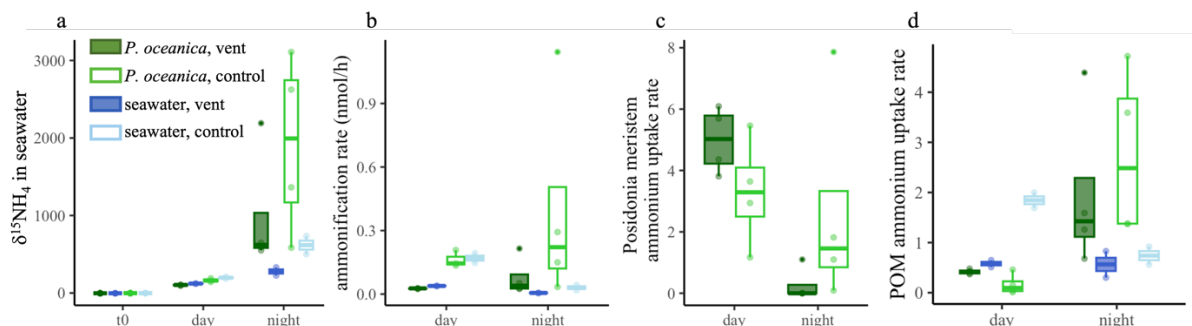


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711 Figure 1. Carbon uptake rates by a. *P. oceanica* and b. seawater. In a, carbon fixation or
712 respiration, in mg C per hour per g *P. oceanica* dry mass in incubation bottles during daylight
713 and nighttime hours. *P. oceanica* carbon fixation was based on oxygen change, following
714 subtraction of water column rates. *P. oceanica* carbon fixation was greater during the day, but
715 did not differ between control and vent sites. Two-way ANOVA (site effect 0.646, diel effect
716 $p < 0.001$, interaction $p = 0.728$). In b, carbon uptake by unfiltered seawater (in mg C per L per
717 hour) is shown for both sites and in incubation bottles with and without *P. oceanica*. POM
718 carbon uptake differed between day and night ($p < 0.001$) and with *P. oceanica* ($p = 0.002$); there
719 were significant interactions with time of day and locale ($p = 0.004$) and time of day and treatment
720 ($p < 0.001$). Boxplots show mean values and delimit 25th and 75th percentiles; all points are
721 shown, statistical tests in Table S2.

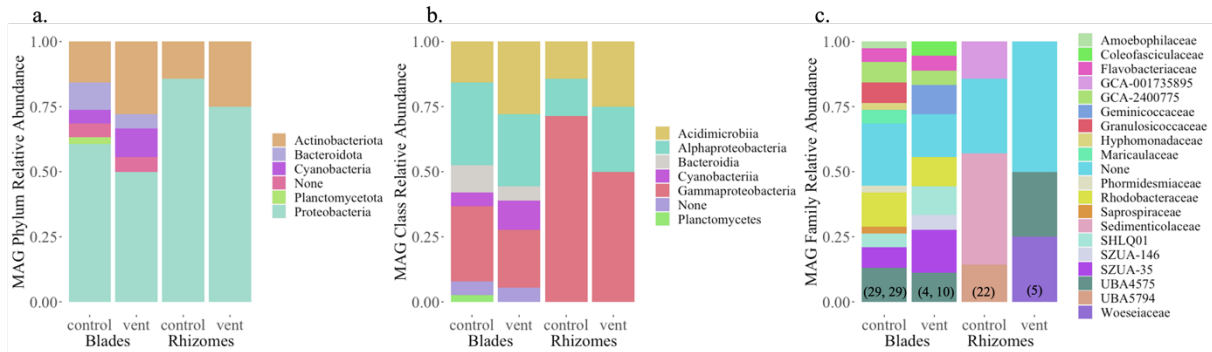
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Figure 2

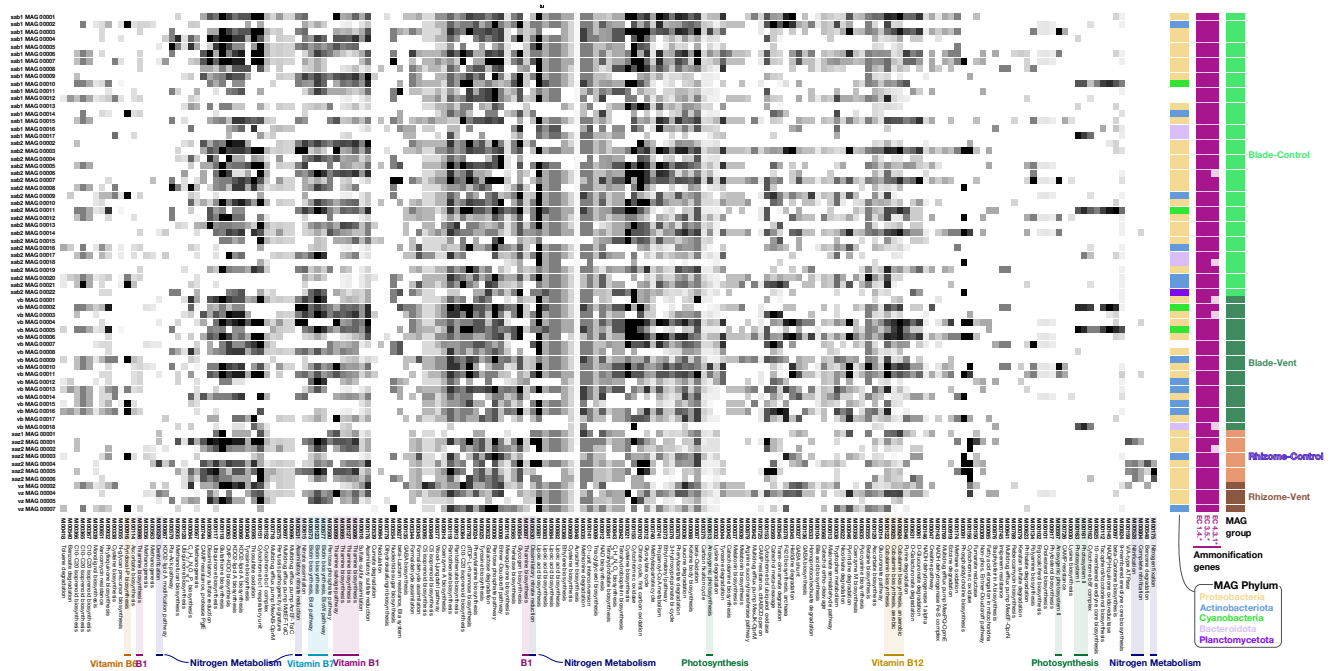


724
 725 Figure 2. The fate of ^{15}N amino acids added to incubation bottles with and without *P. oceanica*.
 726 In a, $\delta^{15}\text{N}$ ammonium (‰) in seawater in incubation bottles with *P. oceanica* or seawater only. A
 727 comparison of the values during the end of day census and the end of experiment census, showed
 728 a significant increase in the bottles at the end of the night, with increased values at SA and with
 729 *P. oceanica*. There was also an interaction between time and treatment; the increase with time
 730 was highest with *P. oceanica*. In b. The ammonification rate in nmol per hour across the two
 731 locales and attributed to *P. oceanica* and seawater versus seawater only based on equation (1).
 732 The control site had greater ammonification compared with the vent sites ($p=0.002$) and *P.*
 733 *oceanica* was nearly associated with greater ammonification rates than seawater ($p=0.060$), while
 734 rates did not differ overall between night and day ($p=0.732$). Examining *P. oceanica* separate
 735 from seawater during day versus night, *P. oceanica* has no diel pattern, while the POM in
 736 seawater is associated with greater ammonification during the day, perhaps due to the greater
 737 supply of DOC. In c, ammonium uptake rate in $\mu\text{mol N per g dry mass per hour}$ in the *Posidonia*
 738 meristem during both day and night periods using equation (2). Uptake was greater during the
 739 day ($p=0.002$), with a significant interaction ($p=0.020$), indicating a relatively greater uptake of
 740 ^{15}N ammonium at night in the control site. In d., the uptake of ammonium by POM was greater
 741 when *P. oceanica* is absent from the incubation bottles during the day, and this effect was
 742 greatest in the control SA site, based on the interaction between locale and treatment ($p=0.033$).
 743 At night, the situation is reversed and the incubation bottles with *P. oceanica* resulted in a greater
 744 incorporation of ^{15}N into the POM in the seawater compared with bottles that had no *P. oceanica*
 745 ($p=0.013$). Statistical analyses are in Table S2.

747
748 Figure 3
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751
752 Figure 3. The taxonomic assignments of 67 metagenome-assembled genomes (MAGs) in the 6
753 samples from the blade or rhizome surface of *Posidonia* as a. Phylum, b. Class and c. Family.
754 There were 22 high quality MAGs and 42 medium quality MAGs, while taxonomy was not
755 found for 3. In c, alpha diversity in each sample is shown and based on ‘anvi-estimate-scg-
756 taxonomy’. See Supplemental Table S4 and S5.
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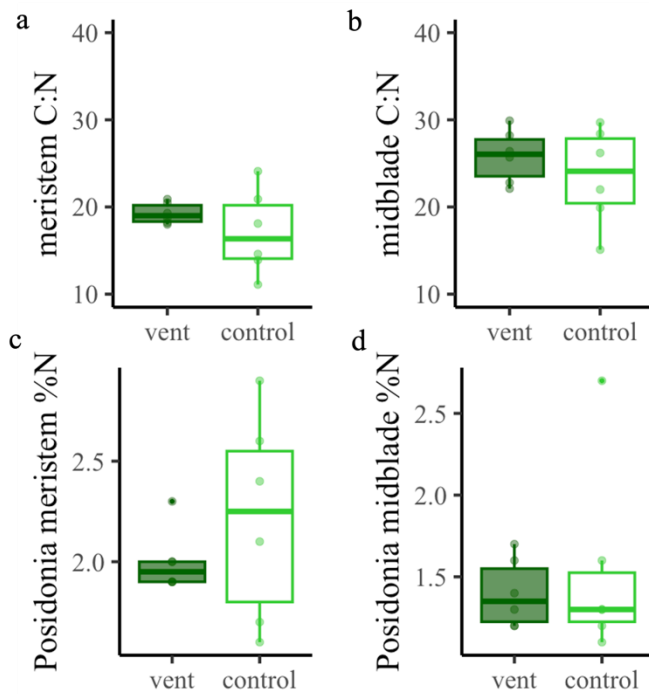
760
761 Figure 4. The metabolisms in 67 microbial metagenome assembled genomes (MAGs) assembled
762 on *P. oceanica* at Ischia, Italy across 5 Phyla and on four different tissue types. The completion
763 (black=complete, white=absent) in each microbial MAG for metabolic modules that showed
764 differences across many of the MAGs. A full list of MAG metabolisms are in Table S7. Every
765 MAG had some ammonification genes (Table S6). The figure was generated from anvi'o⁸³.

766

767 **Supplementary Figures**

768

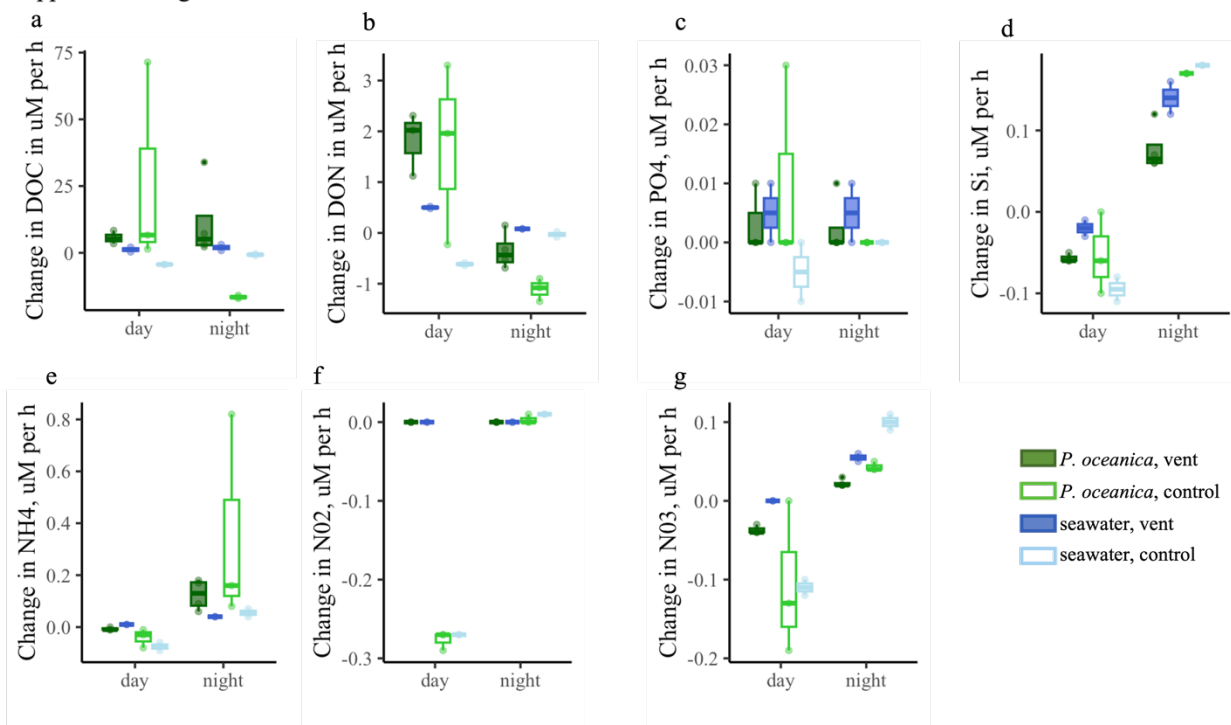
Supplemental Figure 1



769

770 Supplementary Figure 1. *P. oceanica* tissue and POM carbon and nitrogen content. The ratio of
771 tissue carbon to nitrogen did not differ in the a. meristem (n=6, t=1.057, p = 0.334) or b. the
772 midblade region (t = 0.888, p= 0.401), nor were there differences in the overall nitrogen content
773 for either c. meristem or d. midblade. All data in Table S3.

Supplemental Figure 2



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Supplementary Figure 2. Patterns of organic and inorganic nutrients in the incubations throughout the experiment, quantified as the change in uM per hour. Initial values are in Table S1.

Supplementary Tables 1-7 are combined in a single Excel file.